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The toxicity of toxaphene was not influenced by variations in pH or water hardness in static tests. Temperature seemed to influence rate of onset rather than degree of response. Toxicities were similar at 20 and 25 C in flow-through tests, however, a decrease in the time required for mortality to become asymptotic with time was observed. Time-independence was reached after 7 to 16 days of exposure at 25 C while 24 to 34 days were required at 20 C.

The life stage of channel catfish most sensitive to toxaphene poisoning was the swim-up fry with a 96-h LC50 of 0.8 ug/1. Early yolk sac fry were extremely resistant with a 24-h LC50 of 4.7 mg/1, however, within 96 hours the yolk had adsorbed and the LC50 had declined to 8.0 ug/1.

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ACUTE TOXICITY OF TOXAPHENE TO FATHEAD MINNOWS, CHANNEL CATFISH, AND BLUEGILLS

by

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FOREWORD

This report describes the toxicity of toxaphene, a widely used insecticide, to three species of fish, fathead minnows, channel catfish, and bluegills. Research compared the acute toxicity of static tests with continuous flow exposure. Also studied was the importance of various life stages of aquatic organisms when exposed to a chemical pollutant. Environmental variables, pH, water hardness, and temperature were studied under laboratory test conditions to determine their effects on toxaphene toxicity.

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ABSTRACT

Toxaphene was very toxic to fathead minnows (Pimephales promelas), channel catfish (Ictalurus punctatus), and bluegills (Lepomis macrochirus) in static tests; 96-h LC50's ranged from 2.6 to 20 ug/l at 20 C. Fathead minnows were the least susceptible and bluegill and channel catfish were about equal in susceptibility. Prolonged exposures of 12 to 34 days in flow-through tests produced time-independent LC50 values of 0.6 to 1.9 ug/l, significantly lower than the 96-h values.

The toxicity of toxaphene was not influenced by variations in pH or water hardness in static tests. Temperature seemed to influence rate of onset rather than degree of response. Toxicities were similar at 20 and 25 C in flow-through tests, however, a decrease in the time required for mortality to become asymptotic with time was observed. Time-independence was reached after 7 to 16 days of exposure at 25 C while 24 to 34 days were required at 20 C.

The life stage of channel catfish most sensitive to toxaphene poisoning was the swim-up fry with a 96-h LC50 of 0.8 ug/l. Early yolk sac fry were extremely resistant with a 24-h LC50 of 4.7 mg/l however, within 96 hours the yolk had absorbed and the LC50 had declined to 8.0 ug/l.

This report was submitted in partial fulfillment of Contract No. EPA-IAG-141(D) by the Fish-Pesticide Research Laboratory under the sponsorship of the U.S. Environmental Protection Agency. This work was completed as of April, 1976.

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SECTION 1

INTRODUCTION

Within recent years a general concern has developed among scientists about the use of persistent pesticides that are becoming more abundant and widespread in the environment. This concern has induced an increased awareness and closer scrutiny of the effects these chemicals may impose on organisms in the aquatic environment. As more chemicals face either registration cancellation or increased restrictions on their use, pesticide users are forced to depend more and more on fewer alternative chemicals. As a consequence, these chemicals are receiving greater attention from researchers and many are being found to produce detrimental environmental effects.

One compound, toxaphene, has replaced many of the agricultural applications of DDT, for which registration has been canceled. This use has resulted in an increased environmental distribution of toxaphene, and it has been identified as a residue in water and aquatic organisms in various studies (1-4). Toxaphene, like many of the organochlorine chemicals, is persistent (5-9) and highly toxic to fish and other aquatic organisms (10-16). Because of its toxicity to fish, toxaphene was proposed and used as a fish eradicant (17-23). However, the treated waters often remained highly toxic throughout a detoxification time of a few weeks to several years (7, 8, 24-26). This slow detoxification rate led to the eventual elimination of toxaphene as a piscicide, but only after a number of lakes were contaminated with its residues.

Recent data from the National Pesticide Monitoring Program indicate that toxaphene is becoming a more common pollutant in the aquatic environment (D. Walsh, personal communication), thereby increasing the likelihood of its occurrence in diverse habitats and during different stages of a fish's life cycle. The literature contains few data on the effects of environmental variables on toxaphene toxicity or on the susceptibility of different life stages (10). We therefore undertook the present study with the following objectives: (1) to compare the susceptibilities of bluegills, channel catfish, and fathead minnows to toxaphene; (2) to compare the toxicity of toxaphene to these species under static and flow-through test conditions; (3) to determine the effects of temperature, pH, and water hardness on the toxicity of toxaphene to channel catfish; and (4) to compare the susceptibilities of different life stages of channel catfish to toxaphene.

SECTION 2

CONCLUSIONS

Toxaphene is highly toxic to fingerling bluegills, channel catfish, and fathead minnows in static toxicity tests, as evidenced by 96-h LC50's ranging from 2.6 to 20 ug/1 at 20 C and from 2.4 to 23 ug/1 at 25 C.

Time-independent LC50 values (indicating the concentrations where mortality becomes asymptotic with time) for all three species were in the range of 0.6 to 1.9 ug/1.

Water hardness and pH did not appreciably alter toxaphene toxicity.

Temperature appeared to hasten the rate of onset but not the degree of response. For an equivalent response fish required a longer time at 20 C than at 25 C.

In channel catfish, early swim-up fry were the most susceptible life stage (96-h LC50, 0.8 ug/1); yolk-sac fry, before yolk absorption, were the least susceptible (24-h LC50, 4.7 mg/1).

SECTION 3

RECOMMENDATIONS

The 96-h LC50 should be used in preference to the lethal threshold concentration when calculating application factors or maximum acceptable toxicant concentrations.

Thorough studies of the acute toxicity of toxicants to different life stages of fish species and the influence on toxicity by environmental variables such as temperature, pH, and hardness should be performed when "safe" levels of pollutants are being estimated from acute toxicity data.

SECTION 4

METHODS AND MATERIALS

Channel catfish (Ictalurus punctatus), and bluegills (Lepomis macrochirus) used in the toxicity tests were obtained from National fish hatcheries as fingerlings (weight range, 0.5 to 1.5 g). Fathead minnows (Pimephales promelas) were reared in ponds from stock obtained from the Environmental Research Laboratory, Duluth, Minnesota. Although the minnows were in the same size range, they were immature adults rather than fingerlings. Early life stages of channel catfish were reared from eggs produced at the Fish-Pesticide Research Laboratory. Fish used in any single test were of the same lot and were held in laboratory facilities similar to those described by Brauhn and Schoettger (27) for at least 14 days before testing. Holding facilities were supplied with 17 C well water with a pH of 7.4 and alkalinity and total hardness of 237 and 272 mg/l (as $CaCO_3$), respectively. Channel catfish eggs were hatched and the fry reared at 25 C.

Toxicity tests were conducted according to the procedures outlined in American Public Health Association (28) and Committee for Methods for Toxicity Tests with Aquatic Organisms (29). Basic static tests were conducted in reconstituted water with a pH of 7.4 and alkalinity and total hardness of 35 and 40 mg/l ($CaCO_3$), respectively. The influence of water quality was investigated by measuring toxicity at various pH's and water hardnesses. Appropriate buffers and salt mixtures were used to attain the desired values while maintaining other variables constant (29, 30). Water quality values were measured prior to each test to assure that they were within the recommended range of values. Tests were conducted at temperatures of 15, 20, and 25 C and maintained (± 1 C) by constant temperature water baths. Flow-through tests were conducted in the well water described above using a proportional diluter system modeled after Mount and Brungs (31) and modified as recommended by McAllister et al. (32). The dilution ratio between test concentrations was 0.75. Well water used in the flow-through tests was first passed through an ultraviolet light sterilizer. Ten fish per concentration were used in static tests and 30 per concentration in flow-through tests; at least 8 concentrations were used in each test.

Technical toxaphene used in this study was supplied by Hercules, Incorporated, Wilmington, Delaware. It is described as being chlorinated camphene containing 67 to 69% chlorine and is considered to be 100% active ingredient (33). Stock solutions were prepared by dissolving the required amount of chemical in acetone. Test concentrations were accomplished by the addition of an appropriate amount of the stock solution to a test vessel. Amounts of acetone to water were 1 ml/l or less. All concentrations were based on the

total amount of chemical used and all data are expressed as concentrations of total material. In flow-through tests, concentrations delivered to each test vessel were determined by dye calibration of the diluter system. All concentrations are expressed as nominal rather than measured values.

Toxicity values are expressed as LC50's (estimated concentration lethal to 50% of the test population) and were calculated according to the log-probit method of Litchfield and Wilcoxon (34). Values were considered significantly different when the 95% confidence limits about the LC50's did not overlap (28). If less than 50% of the organisms died in the highest concentration, the LC50 for that test was stated as being greater than that concentration. Time-independent lethal concentrations (TILC50-concentration estimate at which 50% of the test population can survive for an indefinite period of exposure) were calculated by the method of Green (35). For these determinations, except for the bluegill test at 25 C, exposures were continued for 30 days or until total mortality over a 4 day period was 5% or less of the test population and the daily mortality in any single concentration did not exceed 10% of the initial number of animals per concentration.

SECTION 5

RESULTS

Static toxicity tests showed that fathead minnows were the least sensitive to toxaphene and bluegills and channel catfish were about equal in susceptibility (Table 1). The 96-h LC50 of 20 ug/l for fathead minnows at 20 C was 7.7 times the value for bluegills and 4.8 times that for channel catfish. The greater resistance of the fathead minnows, however, may have been a consequence of the difference in maturity between them and the bluegills or channel catfish. An increase in test temperature from 20 to 25 C did not appear to alter the toxicity for any of the three species.

After continuous exposure in flow-through tests at 20 C, the 96-h LC50 of 7.0 ug/l observed for fathead minnows was about one-third that observed in static tests (Table 2). Such a large within species difference was not apparent between the two test methods in bluegills or channel catfish. When exposures were continued until response became asymptotic with time, LC50 values decreased to one-half or less of the 96-h values; the estimated TILC50's were 0.6 ug/l for bluegills, 1.0 ug/l for channel catfish, and 1.8 ug/l for fathead minnows.

Although differences in response were observed, the changes in LC50's that occurred in flow-through tests when temperatures were increased from 20 to 25 C were irregular and relatively small. It was noted, however (except in the test with bluegills where system failure forced early termination of the test), that the tests at 25 C reached time-independence in response at an earlier date (7 to 16 days) than those at 20 C (24 to 34 days) (Figs. 1-3). Even so, the TILC50's for all species and temperatures tested were similar.

The lethality of toxaphene to channel catfish did not appear to be altered by pH, water hardness, or temperature in static tests (Table 3). The 96-h LC50's were not materially changed when temperatures varied from 15 to 25 C, pH from 6.5 to 8.3, or total hardness from 10 to 320 ug/l ($CaCO_3$). An exception was the 24-h LC50 of 12.5 ug/l at 15 C, which was considerably higher than the values at 20 and 25 C.

A substantial difference in susceptibility was observed between early life stages of channel catfish. Newly hatched fry, before absorption of the yolk, were extremely resistant to toxaphene poisoning as indicated by the 24-h LC50 of 4700 ug/l (Table 4). However, the toxicity sharply increased as the yolk was observed. After 96 hours, when all yolk had been absorbed, the LC50 decreased to 8.0 ug/l. This increase in susceptibility continued to the swim-up fry stage where LC50's were 4.0 ug/l at 24 hours and 0.8 at 96 hours. Thereafter, resistance increased; the swim-up fry were the most sensitive life stage.

TABLE 1. ACUTE TOXICITY OF TOXAPHENE TO THREE FISHES AT TWO TEMPERATURES IN STATIC TOXICITY TESTS.

(ug/1)

Species[a]	20 C		25 C	
	24 h	96 h	24 h	96 h
Fathead minnow	24 (20-28)	20 (15-27)	23 (18-29)	23 (18-29)
Channel catfish	7.8 (6.3-9.6)	4.2 (2.9-6.0)	6.4 (4.9-8.4)	3.7 (2.3-6.0)
Bluegill	6.8 (6.2-7.4)	2.6 (2.2-3.0)	6.6 (5.5-7.4)	2.4 (2.1-2.7)

[a]Fish size ranged from 0.5 to 1.5 g.

TABLE 2. ACUTE TOXICITY OF TOXAPHENE TO THREE FISHES AT TWO TEMPERATURES IN FLOW-THROUGH TOXICITY TESTS.

(ug/1)

Species[a]	Temp (°C)	LC50 values (and 95% confidence limits) 4 days	8 days	12 days	16 days	20 days	24 days	Terminal value Day	Terminal value LC50	TILC50
Fathead minnow	20	7.0 (6.3-7.7)	4.3 (3.8-4.9)	3.6 (3.3-3.9)	2.9 (2.7-3.1)	2.7 (2.5-2.9)	2.6 (2.5-2.7)	24	2.6 (2.5-2.7)	1.8
	25	5.0 (3.4-7.4)	2.1 (1.5-3.0)	1.8 (1.3-2.3)	1.5 (1.0-2.1)			16	1.5 (1.0-2.1)	1.0
Channel catfish	20	5.5 (4.9-6.2)	4.4 (4.0-4.9)	3.2 (2.9-3.5)	2.9 (2.6-3.2)	2.5 (2.2-2.8)	2.1 (1.9-2.3)	29	1.9 (1.7-2.1)	1.0
	25	7.5 (6.0-9.4)	3.7 (3.0-4.5)	3.7 (3.0-4.5)				12	3.7 (3.0-4.5)	1.9
Bluegill	20	4.7 (4.4-5.0)	2.1 (1.9-2.3)	1.4 (1.3-1.5)	1.2[b]	0.9[b]	0.8 (0.6-0.8)	34	0.7	0.6
	25	3.4 (3.0-3.8)						7	1.4 (1.2-1.7)	

[a]Fish size ranged from 0.5 to 1.5 g except for channel catfish which were 4 g in the 20 C test and 0.15g in the 25 C test.

[b]Data inadequate to calculate confidence limits.

TABLE 3. INFLUENCE OF TEMPERATURE, pH, AND WATER HARDNESS ON THE ACUTE TOXICITY OF TOXAPHENE TO FINGERLING CHANNEL CATFISH[a] IN STATIC TESTS.

(ug/l)

Test Conditions				LC50 values (and 95% confidence limits)	
Temp (°C)	pH	Alkalinity (mg/l)	Hardness (mg/l)	24 h	96 h
15	7.4	35	40	12.5 (9.7-16.1)	4.7 (3.2-6.8)
20	7.4	35	40	7.8 (6.3-9.6)	4.2 (2.9-6.0)
25	7.4	35	40	6.4 (4.9-8.4)	3.7 (2.3-6.0)
20	6.5	35	40	6.2 (3.9-10.0)	2.7 (1.9-3.8)
20	7.5	35	40	7.9 (5.9-10.5)	3.4 (2.5-4.6)
20	8.3	35	40	7.5 (6.3-9.0)	3.0 (2.1-4.3)
20	8.2	220	10	5.6 (4.2-7.5)	3.9 (3.1-4.8)
20	8.2	220	40	6.7 (5.2-8.6)	3.2 (2.3-4.4)
20	8.2	220	160	6.1 (4.6-8.0)	3.9 (2.0-5.1)
20	8.2	220	320	7.3 (5.8-9.3)	4.7 (3.7-5.9)

[a]Fish size ranged from 0.5 to 1.5 g.

TABLE 4. THE ACUTE TOXICITY OF TOXAPHENE TO THREE LIFE STAGES OF CHANNEL CATFISH IN STATIC TESTS AT 25 C.

(ug/l)

Life stage[a]	LC50 values (and 95% confidence limits)	
	24 h	96 h
Yolk-sac fry	4700 (3040-7260)	8.0 (6.0-10.6)
Swim-up Fry	4.0 (3.2-5.0)	0.8 (0.5-1.2)
Fingerling	7.0 (5.4-9.1)	2.8 (2.0-4.0)

[a]Yolk-sac fry were 1 to 4 days old, swim-up fry were 5 to 8 days old, and fingerlings size ranged from 0.5 to 1.5 g.

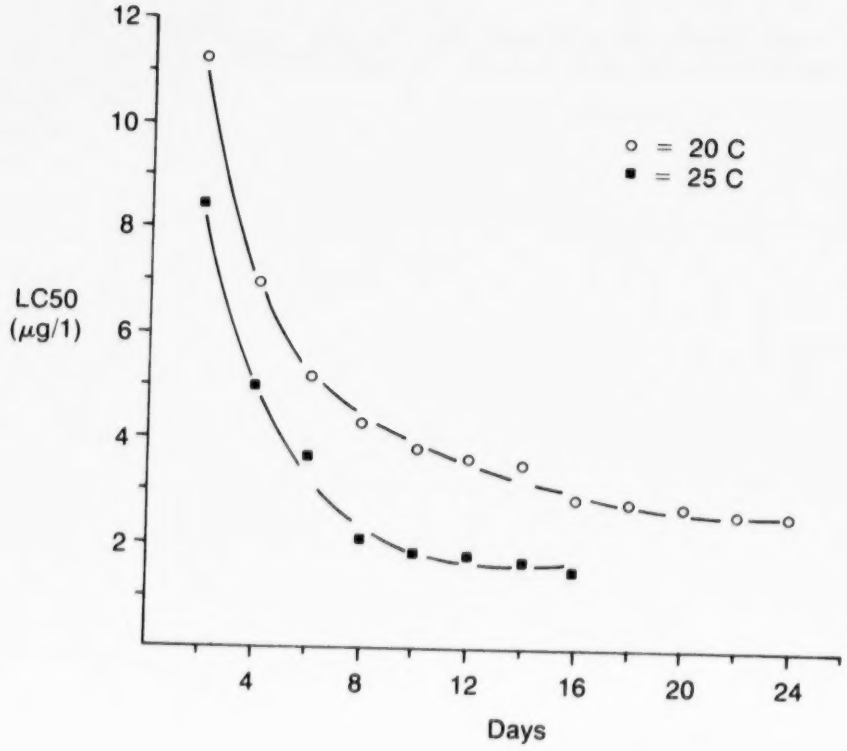

Figure 1. Response curve for fathead minnows exposed to toxaphene in flow-through toxicity tests.

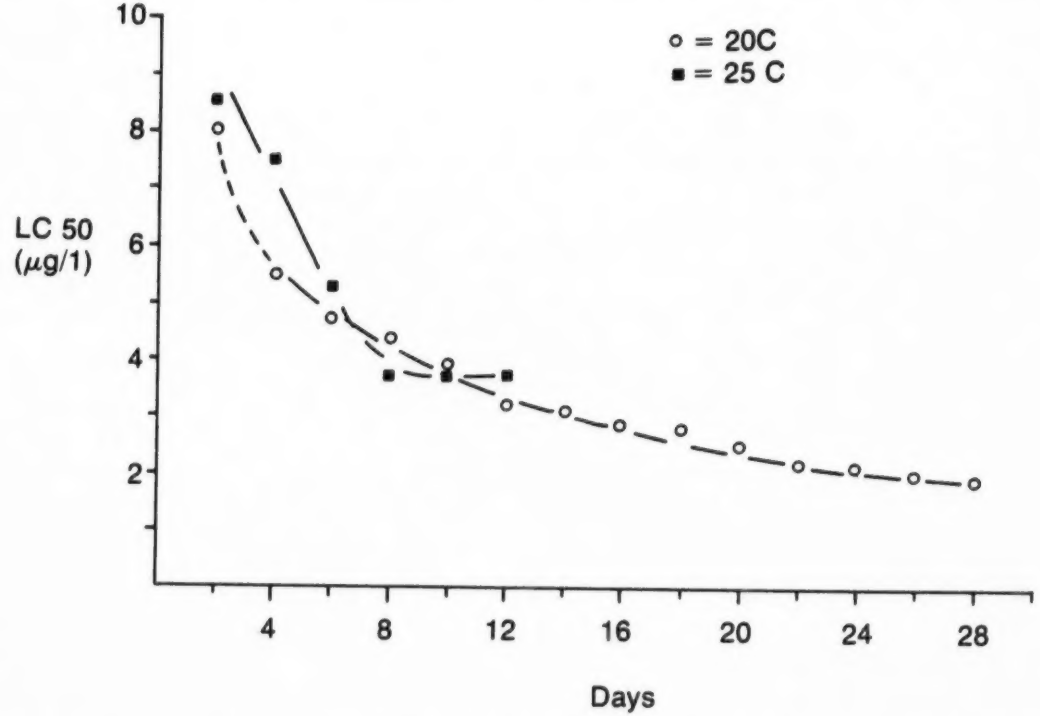

Figure 2. Response curve for channel catfish exposed to toxaphene in flow-through toxicity tests. Broken line connects estimated value for 2-d LC50 at 20 C.

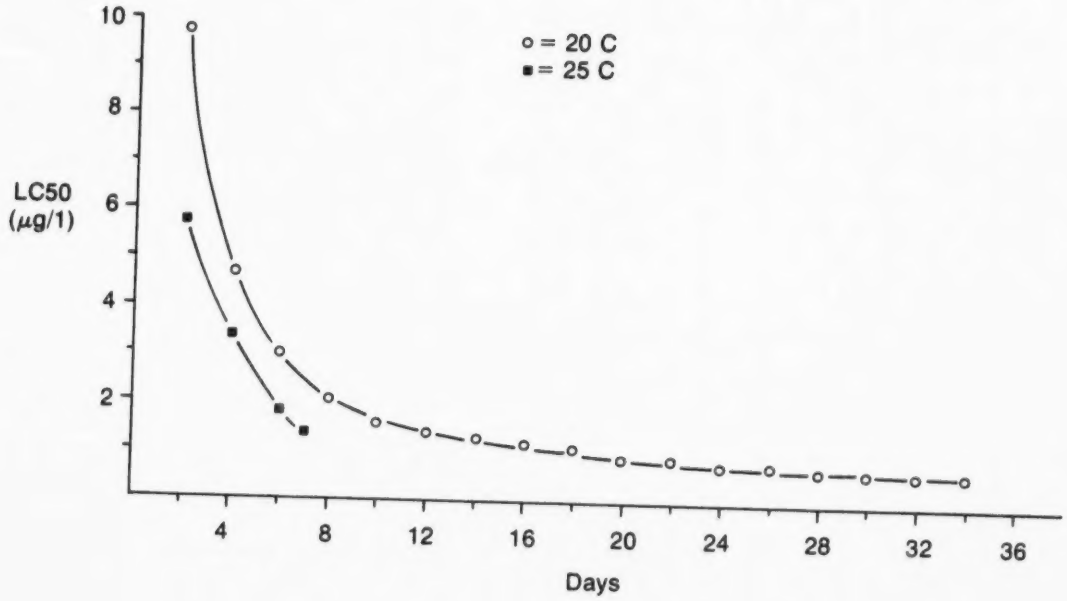

Figure 3. Response curve for bluegills exposed to toxaphene in flow-through toxicity tests.

SECTION 6

DISCUSSION

Toxaphene, one of the more toxic of the organochlorine insecticides to fish (12), has a lethal range of concentrations reported to be between 5 and 100 ug/l for most freshwater species (36). Data on similar species from the Fish-Pesticide Research Laboratory (37) show a range of 96-h LC50's from static toxicity tests of 2 to 23 ug/l, the upper limit for lethality being lower than previously reported. Such differences in LC50 values, however, could be due to variations in test methods and precision rather than to toxicity limits. Data from the present study are in general agreement with ranges reported for bluegills, channel catfish, and fathead minnows (38).

In comparison with the static tests, exposure of bluegills and channel catfish to toxaphene in flow-through test systems did not produce an appreciable increase in toxicity. In contrast, fathead minnows were notably more susceptible to toxaphene poisoning in the flow-through system, thereby eliminating the differences in susceptibility observed in static tests; in effect, LC50's were similar for all three species. The similarity in response was maintained throughout the prolonged exposures and the terminal values were all within the range of 0.7 to 3.7 ug/l.

Time-independent LC50's were similar for all three species, although the rate at which response became asymptotic with time varied considerably. TILC50 values are of interest because they represent the theoretical concentration at which 50% of the test population can survive toxicant exposure for a prolonged period (39). Presumably, the surviving animals are sufficiently able to handle these concentrations of chemical, through detoxification, metabolism, or other means, to the extent that the imposition of mortality on the test population due to the toxicant exposure is no longer significant. In this context, the toxicant concentration should represent the range where the response is no longer acute and becomes chronic in nature.

It has been suggested by Eaton (40) that the lethal threshold concentration (LTC) is the more appropriate value to use in the calculation of application factors as defined by Mount (41). He stated that it provides better acute values to use with experimentally determined application factors when computing "maximum acceptable toxicant concentrations" (MATC) for species for which only acute toxicity data are available. He identified the LTC value as the point in an acute toxicity test at which the mortality rate is 10% or less of the original number of fish in any concentration during the preceeding 24 hours. Using this criterion, LTC values determined for our (20 C) tests were 4.3, 4.5, and 2.0 ug/l for fathead minnows, channel catfish,

and bluegills, respectively. When compared with the 4 day LC50's (Table 2), the values are not greatly different for these species and would produce relatively small differences in calculated MATC values. If the definition of an application factor presented by the National Academy of Sciences (38) is used (the numerical value for the ratio of the safe concentration to the acutely lethal concentration), it would seem that the 96-h LC50 would be as acceptable as the LTC. Since, as a general rule, 96-h LC50's would be less expensive and time consuming to generate and there are broadly accepted, standardized procedures for conducting 96-h toxicity tests (29), we recommend the continued use of the 96-h LC50 for calculating MATC values when experimentally determined values are not available. This would maintain continuity with the National Academy of Sciences (38) since it uses the 96-h LC50 in generating calculated MATC values.

If the arbitrary value of 0.01 is used as the application factor for toxaphene (38), then the calculated values of the MATC's for bluegills, channel catfish, and fathead minnows are 47, 55, and 70 ng/l, respectively. From a chronic toxicity study with brook trout (Salvelinus fontinalis), Mayer et al. (42) reports that the MATC is below 39 ng/l and recommended that the water concentration of toxaphene be below this value to protect this species. Our data, however, indicate that this concentration may be adequate for the protection of these three species.

Of the environmental variables studied, only temperature effected the acute toxicity of toxaphene. Considering the chemical stability of toxaphene, it would be expected that pH and water hardness, under laboratory test conditions, would have little effects on toxicity. Although irregular in nature, it appeared that higher temperatures increased the rate at which the response occurred and a decrease in the time required for mortality to become asymptotic with time was observed in the flow-through tests conducted at 25 C. Such an influence on response time has previously been reported by Hussein et al. (43) and Kopp (13). An explanation of these results is not immediately apparent. Macek et al. (44) have suggested that rates of uptake and enzyme activity may offer at least partial explanations of these data.

Variations in the susceptibility of different life stages appeared to be a factor of major importance. Although the early yolk-sac fry were highly resistant to toxaphene poisoning, susceptibility sharply increased after the yolk was absorbed. We believe that the resistance of the yolk-sac fry may be correlated with the lipid-solubility of toxaphene and that the yolk is a reservoir in which toxaphene is sequestered. Data reported by Mayer et al. (42), which indicated that brook trout fry accumulated toxaphene up to 76,000 times the water concentration within 15 days after hatching, appear to support this view. As yolk absorption advanced, toxaphene residues would be mobilized and mortality would increase.

It was not surprising that swim-up fry were the most sensitive stage studied. They were in the phase where feeding and movement were being initiated and embryonic differentiation was being completed. It would be expected that an organism undergoing the stress of such physiological change would be highly susceptible to externally superimposed stress. Significantly, our data show that the 96-h LC50 for swim-up fry is equivalent to the as-

ymptotic LC50 for fingerlings. Such data point out the importance of studying various life stages of aquatic organisms as well as various species when "safe" concentrations of pollutants are being determined.

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